Research Article

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Sublethal and transgenerational effects of thiamethoxam applied to cotton seed on *Chrysoperla externa* and *Harmonia axyridis*

Rafaella R Sâmia,[a] Pablo C Gontijo,[b*] Rodrigo L Oliveira[a] and Geraldo A Carvalho[a]

Abstract

BACKGROUND: Thiamethoxam, when applied as a seed treatment, can contaminate plant products, such as extrafloral nectar, and have non-target effects on beneficial arthropods. This study assessed the non-target effects of thiamethoxam applied to cotton seed on the life history parameters of the predators *Chrysoperla externa* (Neuroptera: Chrysopidae) and *Harmonia axyridis* (Coleoptera: Coccinellidae).

RESULTS: Exposure of *C. externa* larvae to plants grown from thiamethoxam-treated seeds caused sublethal and transgenerational effects. Thiamethoxam treatment doubled the proportion of pharate adults and reduced egg fertility in *C. externa* F0 and F1 generations. In addition, the insecticide prolonged pupal developmental time in the *C. externa* F1 generation. Thiamethoxam treatment also had a transgenerational effect on exposed *H. axyridis* larvae, reducing pupal survival in the F1 generation. In the adult bioassay, thiamethoxam treatment reduced egg fertility of *C. externa*, prolonged the larval period, and reduced both fecundity and egg fertility of the F1 generation. Thiamethoxam also caused transgenerational effect on *H. axyridis* adults, reducing larval survival of the F1 generation.

CONCLUSION: Thiamethoxam seed treatment was harmful for both predators, but *C. externa* was more affected by the insecticide than *H. axyridis*.

Keywords: non-target organism; neonicotinoid; systemic insecticide; environment risk assessment; green lacewing; lady beetle

1 INTRODUCTION

Neonicotinoids are the most widely used class of insecticides worldwide, with an estimated market value of US$ 4650 million.[1] Neonicotinoids are classified by the Insecticide Resistance Action Committee (IRAC) as subgroup 4A,[2] acting as an agonist of nicotinic acetylcholine receptors in the central nervous systems of insects.[3] Since their introduction in the 1990s, the use of neonicotinoids has increased dramatically, driven by the large-scale deployment of seed treatments on field crops such as maize, soybean, wheat and cotton.[4] Neonicotinoids are successful as a seed treatment because they act systemically within plant vascular tissues, given their low lipophilicity, typically exposing a low octanol–water partition coefficient (log P_{oct}).[5]

The neonicotinoid class has seven unique compounds; thiamethoxam a second-generation neonicotinoid, was first synthesized in 1991 but only marketed in 2013.[6] Thiamethoxam has broad-spectrum insecticidal activity and offers excellent control of a wide variety of pests in many crops.[7] In cotton fields, seeds treated with thiamethoxam provide efficient control of important early-season sucking insect pests, such as tobacco thrips *Frankliniella fusca* Hinds (Thysanoptera: Thripidae),[8] cotton aphids *Aphis gossypii* (Hemiptera: Aphididae)[9] and leafhoppers *Amrasca devastans* (Distant) (Hemiptera: Cicadellidae).[10] According to Zhang *et al.*[11] cotton seed treated with thiamethoxam is effective against whitefly *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae) biotype B for up to 45 days under laboratory and greenhouse conditions, and for up to ~ 2 months under field conditions. Furthermore, thiamethoxam seed treatment can enhance the growth of cotton seedlings under heat stress.[12]

Although many insect pests are successfully controlled by neonicotinoids, use of these insecticides as a seed treatment has been linked to environmental contamination risks and can have non-target effects on beneficial arthropods, especially pollinators.[13–17] Beneficial arthropods may be exposed to systemic insecticides such as thiamethoxam via the direct consumption of pollen and floral and extrafloral nectar (EFN) from plants grown from treated seeds and via other routes.[5,13,18,19] Production of EFN is known to be an indirect plant defense against herbivorous arthropods, increasing plant attractiveness to natural enemies[20] that use EFN as a dietary complementation, in either the presence or absence of prey.[21,22] In cotton

* Correspondence to: PC Gontijo, School of Agronomy, Federal University of Goiás, Goiânia, GO 74.690-900, Brazil. E-mail: pablocgontijo@gmail.com

a Department of Entomology, Federal University of Lavras, Lavras, Brazil

b School of Agronomy, Federal University of Goiás, Goiânia, Brazil

plants, extrafloral nectaries are located under the leaves, in the largest midribs, on the squares between the bracts, and at the bases of bracts,[23,24] easily accessible to beneficial insects.[25]

The natural enemy community in the cotton ecosystem is very diverse.[26,27] Among the many beneficial predators in cotton crops, green lacewings *Chrysoperla externa* (Hagen) (Neuroptera: Chrysopidae) and lady beetles *Harmonia axyridis* (Pallas) (Coleoptera: Coccinellidae) stand out as polyphagous predators that contribute to the natural control of several pests of economic importance. Lacewings and lady beetles consume pollen, nectar and EFN during both larval and adult stages.[28–30] Therefore, given the risk of cotton EFN contamination by neonicotinoids, the objective of this study was to assess the non-target effects of thiamethoxam on the life-history parameters of *C. externa* and *H. axyridis* that consumed cotton EFN of plants grown from treated seeds during both larval and adult stages. This information can help to better understand the potential risks to beneficial arthropods of systemic insecticides used in seed treatments.

2 MATERIALS AND METHODS

2.1 Insect colonies

Adults of *C. externa* and *H. axyridis* were collected from vegetable fields at the Federal University of Lavras, Lavras, Minas Gerais, Brazil (21°13′48.12′′S; 44°58′56.32′′W). All insect colonies and bioassays were performed in a climate-controlled room held at 25 ± 2 °C, 70 ± 10% relative humidity (RH) and a 12:12 h (L/D) photoperiod. The colony of *C. externa* was maintained in a plastic container (15.0 cm in diameter × 20.0 cm in height) covered with an organdy mesh screen. Lacewing adults were fed an artificial diet composed of honey and brewer's yeast (1:1) as well as water on a piece of sponge, both refreshed every 48 h. Larvae were fed *ad libitum* every 48 h with frozen eggs of *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae). The *H. axyridis* colony was maintained similarly to the lacewing colony; larvae and adults of lady beetles were fed with frozen *E. kuehniella* eggs *ad libitum*. All bioassays were conducted with the second laboratory generation of both *C. externa* and *H. axyridis* predators.

2.2 Cotton plants

Commercial cotton seeds (Bollgard™, Monsanto, São Paulo, SP, Brazil) were treated with thiamethoxam at a rate of 600 mL $100\ kg^{-1}$ of seed (Cruiser® 350 FS, 35 g a.i. L^{-1}, neonicotinoid, suspension concentrate, Syngenta Crop Protection, São Paulo, SP, Brazil). This insecticide rate corresponds to the maximum field concentration recommended on the label by the manufacturer and the Brazilian Ministry of Agriculture for cotton seed treatment.[31] Treated and untreated (control) seeds were sown in plastic pots (200 mL) filled with a mixture of soil and commercial substrate (Plantmax®) (1:1), and were germinated in a greenhouse at 25 ± 3 °C, 70 ± 10% RH, under natural light. Plants were watered every 24 h, sparingly, to avoid excessive leaching of insecticide. All bioassays used plants at the V2 stage (~ 15 days post emergence), when EFN is already present[32] and thiamethoxam residues in cotton leaves are high.[9]

2.3 Larval bioassay

Bioassays of both species were conducted with 10 neonate larvae (1–2 h old) in each replicate ($n = 5$ per treatment). Larvae were caged on a V2 stage cotton plant grown from either thiamethoxam-treated or untreated seeds (control), until larvae pupated.

During the exposure period, cotton EFN was the only source of hydration. Larvae were fed with frozen eggs of *E. kuehniella ad libitum* spread on the floor of the cage and refreshed every 48 h. Emerged adults were sexed and the maximum possible number of pairs in each treatment was established to assess thiamethoxam sublethal effects on reproductive parameters of predators (e.g. preoviposition period, fecundity and fertility). Each pair of *C. externa* (control = 8 and thiamethoxam = 10) and *H. axyridis* (control = 15 and thiamethoxam = 13) was held in a plastic cage (15.0 cm diameter × 20.0 cm height) or Petri dish (5.5 cm diameter), respectively. Pairs of *H. axyridis* were established 8 days after emergence of adults to ensure the sexual maturation of females.[33] After 5 days (mated period), males and females of *H. axyridis* were isolated in new Petri dishes (5.5 cm diameter) so that reproduction could be assessed. Adults of *H. axyridis* were fed with frozen *E. kuehniella* eggs *ad libitum*, with water provided on a small sponge, whereas *C. externa* adults were fed with the artificial diet (as above). Food and water were refreshed every 48 h.

All insects were monitored daily; developmental and reproductive parameters (preoviposition period, fecundity and fertility) were recorded according to Gontijo *et al.*[34] and Moscardini *et al.*[35] Adult survival was monitored until the 10th day of oviposition of each female. Fertility was considered similar to egg hatching and assessed by harvesting eggs from each replicate on the third, fifth and seventh days of oviposition, and isolating them in Petri dishes until hatching.

To evaluate transgenerational effects of thiamethoxam seed treatment on the F1 generation survivors of *C. externa* and *H. axyridis*, 30 neonate larvae of the third and fifth clutches of each species and treatment were individualized in Petri dishes (5.5 cm diameter) and reared as previously described. After emergence of adults, the maximum number of pairs was established in each treatment (controls = 18 and 12, thiamethoxam = 14 and 7, for lacewings and lady beetles, respectively). For both species, preoviposition period, fecundity (number of eggs per female) and fertility reproductive parameters were evaluated until the third clutch of each female.

2.4 Adult bioassay

Adult bioassays were conducted similar to the larval bioassay. For this, 24 males and 24 females (0–24 h old) of *C. externa* and *H. axyridis* were individualized by caging them on a V2 stage cotton plant, grown from either thiamethoxam-treated or untreated seeds, for 8 days. During this period, lacewing and lady beetle adults were fed with the artificial diet (as above) and frozen *E. kuehniella* eggs *ad libitum*, respectively, every 48 h. Cotton EFN was the only source of hydration. After 8 days of exposure to cotton plants, adults of *C. externa* and *H. axyridis* were paired ($n = 24$ per treatment) in a plastic cage (15.0 cm diameter × 20.0 cm height) and Petri dishes (5.5 cm diameter), respectively. After 5 days of mating, lady beetle pairs ($n = 24$) were separated and males and females were isolated in new Petri dishes for assessment of survival and reproductive parameters. Reproductive (e.g. preoviposition period, fecundity and fertility) and developmental parameters of lacewings and lady beetles exposed to cotton plants, as well transgenerational effects, were evaluated as in the larvae bioassay.

2.5 Statistical analysis

All data were subjected to Shapiro–Wilk and Bartlett tests to confirm normal distribution and homoscedasticity, respectively.

Table 1. Life history parameters of *Chrysoperla externa* after larvae fed on cotton extrafloral nectar of plants grown from thiamethoxam-treated seeds

	Larvae exposed (F0)					F1 generation				
	Seed treatment		Stats			Seed treatment		Stats		
Parameter	Untreated	Thiamethoxam	Critical value	df	*P*-value	Untreated	Thiamethoxam	Critical value	df	*P*-value
Larval period										
Survival (%)	91.5 ± 1.1	84.0 ± 1.8	40.0^{b}	5,5	0.42	89.9 ± 3.2	81.5 ± 7.7	1.0^{a}	8	0.34
Development time (d)	10.7 ± 0.1	10.6 ± 0.1	0.8^{a}	18	0.42	11.9 ± 0.1	11.6 ± 0.1	1.4^{a}	8	0.20
Pupal period										
Survival (%)	95.5 ± 0.9	98.0 ± 0.6	44.5^{b}	5,5	0.54	100	100			
Development time (d)	10.8 ± 0.2	10.9 ± 0.1	−0.7^{a}	18	0.50	11.9 ± 0.1 b	12.3 ± 0.1 a	−2.5^{a}	8	0.03
Adults										
Pharate adults (%)	15.8 ± 4.7 b	32.0 ± 7.5 a	−1.8^{a}	18	0.04	8.7 ± 3.9 b	21.6 ± 5.1 a	−2.6^{a}	8	0.03
No. adults emerged	35	28				41	33			
Sex ratio (proportion female)	0.40 ± 0.08	0.54 ± 0.09	0.7^{c}	1	0.41	0.49 ± 0.08	0.55 ± 0.09	0.1^{c}	1	0.79
Reproductive parameters										
Preoviposition period (d)	6.0 ± 0.2	6.3 ± 0.2	29.5^{b}	8,10	0.26	4.1 ± 0.1	4.4 ± 0.1	97.0^{b}	14,18	0.18
Fecundity* (eggs female^{-1})	125.9 ± 10.0	132.5 ± 14.2	−0.4^{a}	16	0.72	55.8 ± 4.3	54.2 ± 4.7	126.0^{b}	14,18	0.83
Fertility (egg hatching %)	89.3 ± 2.1 a	68.3 ± 1.6 b	8.0^{a}	16	0.01	93.8 ± 1.9 a	85.9 ± 2.1 b	2.7^{a}	22	0.01
Survival†(%)										
Female	100	60.0 ± 16.3			0.09^{d}	100	100			–
Male	87.5 ± 12.5	90.0 ± 10.0			1.00^{d}	100	100			–

Means (±SE) followed by different letters within rows were significantly different by (a) *t*-test, (b) Mann–Whitney, (c) chi-square or (d) Fisher's exact test ($\alpha = 0.05$). –, non-analyzed data.
*Number of eggs in 10 clutches or number of eggs in the first three clutches (F1 generation).
†Accumulated survival in the last evaluation day.

Subsequently, data were analyzed using an independent Student's *t*-test. Data that did not meet the assumptions of normality and homoscedasticity were analyzed by the non-parametric Mann–Whitney test. Sex ratio [$\sum$♀/$\sum$ (♀ + ♂)] and survivor proportion (♀ and ♂) were analyzed by Chi-square or Fisher's exact test. All analyses were performed using SigmaPlot 12.5 software[36] with $\alpha = 0.05$.

3 RESULTS

Consumption of cotton EFN of plants grown from thiamethoxam-treated seeds by *C. externa* larvae had significant sublethal and transgenerational effects on lacewings. Thiamethoxam seed treatment doubled the proportion of pharate adults and reduced egg fertility of *C. externa* F0 and F1 generations (Table 1). In addition, thiamethoxam prolonged pupal developmental time of *C. externa* F1 generation (Table 1). Thiamethoxam also had a transgenerational effect on exposed *H. axyridis* larvae, reducing pupal survival of the F1 generation, although other developmental and reproductive parameters were unaffected (Table 2).

In the adult exposure bioassay, thiamethoxam applied to cotton seeds reduced egg fertility in exposed *C. externa* adults (F0), prolonged the larval period, and reduced both fecundity and egg fertility of the F1 generation (Table 3). Thiamethoxam also had a transgenerational effect on exposed *H. axyridis* adults, reducing larval survival of the F1 generation. However, no other life-history parameter of *H. axyridis* was significantly affected by thiamethoxam (Table 4).

4 DISCUSSION

Thiamethoxam had sublethal effects on the exposed generation (F0) and also transgenerational effects on the F1 generation, when both stages (larvae and adults) of *C. externa* were exposed to cotton plants grown from treated seeds. In *H. axyridis*, only transgenerational effects were observed, which reduced pupal and larval survival of the F1 generation of exposed larvae and adults, respectively. Sublethal and transgenerational effects of pesticides are, in many cases, neglected although they may cause stress to communities of beneficial organisms and have a great impact on ecological services.[37–40]

Table 2. Life history parameters of *Harmonia axyridis* after larvae fed on cotton extrafloral nectar of plants grown from thiamethoxam-treated seeds

	Larvae exposed (F0)					F1 generation				
	Seed treatment		Stats			Seed treatments		Stats		
Parameter	Untreated	Thiamethoxam	Critical value	df	*P*-value	Untreated	Thiamethoxam	Critical value	df	*P*-value
Larval period										
Survival (%)	85.1 ± 5.0	81.5 ± 3.5	0.6[a]	8	0.57	60.0 ± 10.9	50.0 ± 8.9	9.0[b]	5,5	0.53
Development time (d)	11.6 ± 0.1	11.8 ± 0.1	−1.5[a]	8	0.18	12.3 ± 0.2	13.2 ± 0.7	−1.2[a]	8	0.28
Pupal period										
Survival (%)	100	100				100 a	79.2 ± 6.2 b	2.5[b]	5,5	0.03
Development time (d)	4.5 ± 0.1	4.5 ± 0.0	−0.2[a]	8	0.81	4.2 ± 0.1	4.5 ± 0.2	−1.3[a]	8	0.23
Adults										
No. adults emerged	40	39				30	19			
Sex ratio (proportion female)	0.48 ± 0.08	0.51 ± 0.08	0.0[c]	1	0.91	0.50 ± 0.09	0.42 ± 0.11	0.1[c]	1	0.81
Reproductive parameters										
Preoviposition period (d)	11.3 ± 0.5	12.2 ± 0.6	−1.2[a]	20	0.25	12.2 ± 0.6	13.3 ± 0.9	−1.1[a]	6	0.31
Fecundity* (eggs female^{-1})	150.4 ± 25.9	153.2 ± 28.7	−0.1[a]	20	0.94	29.0 ± 8.6	33.7 ± 9.4	−0.3[a]	6	0.75
No. clutches laid in 10-days	6.5 ± 0.7	6.2 ± 0.6	0.3[a]	20	0.75					
Fertility (egg hatching %)	72.9 ± 10.4	68.5 ± 12.3	0.3[a]	18	0.79	94.1 ± 4.3	92.3 ± 7.7	0.2[a]	5	0.84
Survival† (%)										
Female	100	100			–	100	100			–
Male	100	100			–	66.7 ± 14.2	57.1 ± 20.2			0.38[d]

Means (±SE) followed by different letters within rows were significantly different by (a) *t*-test, (b) Mann–Whitney, (c) Chi-square or (d) Fisher's exact test ($\alpha = 0.05$). –, non-analyzed data.
*Number of eggs in 10-days or number of eggs in the first three clutches (F1 generation).
†Accumulated survival in the last evaluation day.

The negative effects of thiamethoxam on *C. externa* and *H. axyridis* were likely related to the consumption by both predators of cotton EFN contaminated with the insecticide. Although there may also be some contamination of the insects by exposure through contact with exudates and plant tissues. Visual observations during the insect exposure period confirmed that both larvae and adults of *C. externa* and *H. axyridis* fed on cotton EFN. Contamination of cotton EFN with thiamethoxam occurs due to its systemic property, allowing translocation to all plant tissues.[5,41] According to Zhang *et al.*[9] under field conditions, the concentration of thiamethoxam applied as a seed treatment in cotton leaves is ~ 5.0 mg kg^{-1} in plants within 16 days (post emergence); the plants studied here were of a similar stage.

One explanation for the higher toxicity of thiamethoxam to *C. externa* compared with *H. axyridis* is the higher consumption of cotton EFN by lacewings. Although EFN increases the fitness of both lacewings[42] and lady beetles,[43] lacewings possibly consume a greater amount of EFN due to their hydration needs, especially in adults.[28,44] By contrast, lady beetles show greater drought tolerance, which is likely the key to this species success in arid environments.[35] During insect exposure to treated and untreated plants, no other source of hydration was provided. Limburg and Rosenheim[44] found that larvae of *Chrysoperla plorabunda* (Fitch) (Neuroptera: Chrysopidae) used cotton EFN as a dietary supplement, and that its consumption was reduced by an increased supply of prey. Our results show that *C. externa* larvae feed on cotton EFN even with *ad libitum* availability of prey (*E. kuehniella* eggs), given the sublethal effects caused by thiamethoxam. Gontijo *et al.*[34] also reported that survival and fecundity of *Chrysoperla carnea* (Stephens) were reduced when adults consumed sunflower EFN of plants grown from thiamethoxam-treated seeds. However, in contrast to the results of this study, the authors did not find negative effects of thiamethoxam when *C. carnea* larvae consumed sunflower EFN grown from treated seed and in the absence of prey. This may be related to different sensitivities to thiamethoxam in lacewings species (*C. externa* and *C. carnea*) and to greater ease of access of lacewings to cotton EFNs, in comparison with sunflower plants.

The proportion of pharate lacewings when *C. externa* larvae consumed cotton EFN from thiamethoxam-treated plants was two to three times higher compared with controls in the F0 and F1 generations, respectively. Mechanisms associated with this effect are unclear, because thiamethoxam is an agonist of nicotinic acetylcholine receptors[3] and has no direct action on insect metamorphosis. The availability of prey during the insect exposure period may also justify the hypothesis of lower consumption of

Table 3. Life history parameters of *Chrysoperla externa* after adults fed on cotton extrafloral nectar of plants grown from thiamethoxam-treated seeds

	Parameter	Seed treatment		Stats		
		Untreated	Thiamethoxam	Critical value	df	P-value
Adults exposed (F0)	Preoviposition period (d)	5.0 ± 0.0	5.2 ± 0.2	231.0[b]	22, 24	0.09
	Fecundity*(eggs female^{-1})	114.9 ± 14.8	136.5 ± 9.7	223.0[b]	22, 24	0.37
	Fertility (egg hatching %)	97.7 ± 1.3 a	90.1 ± 2.3 b	2.8[a]	44	<0.01
	Survival†(%)					
	Female	91.7 ± 5.8	95.8 ± 4.2			0.91[d]
	Male	83.3 ± 7.8	70.8 ± 9.5	0.5[c]	1	0.49
F1 generation	Larval period					
	Survival (%)	82.9 ± 4.2	75.7 ± 2.9	1.4[a]	12	0.19
	Development time (d)	11.6 ± 0.1 b	12.0 ± 0.1 a	−2.8[a]	12	0.01
	Pupal period					
	Survival (%)	96.4 ± 2.3	94.3 ± 4.1	24.5[b]	7,7	0.99
	Development time (d)	12.6 ± 0.1	12.5 ± 0.1	1.3[a]	12	0.20
	Adults					
	Pharate adults (%)	10.3 ± 3.9	3.8 ± 2.5	15.5[b]	7, 7	0.23
	No. adults emerged	51	47			
	Sex ratio (proportion female)	0.53 ± 0.07	0.62 ± 0.07	0.5[c]	1	0.50
	Reproductive parameters					
	No. pairs mated	18	21			
	Preoviposition period (d)	4.1 ± 0.1	4.1 ± 0.1			–
	Fecundity*(eggs female^{-1})	54.7 ± 3.2 a	43.4 ± 4.3 b	2.1[a]	37	0.04
	Fertility (egg hatching %)	97.0 ± 0.9 a	91.8 ± 2.9 b	279.0[b]	26, 30	0.03
	Survival†(%)					
	Female	100	100			–
	Male	100	100			–

Means (±SE) followed by different letters within rows were significantly different by (a) t-test, (b) Mann–Whitney, (c) chi-square or (d) Fisher's exact test ($\alpha = 0.05$). –, non-analyzed data.
*Number of eggs in 10 clutches or number of eggs in the first three clutches (F1 generation).
†Accumulated survival in the last evaluation day.

EFN by *H. axyridis* larvae and adults that prefer animal protein. Low consumption of EFN probably contributes to the lower toxicity of thiamethoxam on *H. axyridis*. Olfactometer tests showed that the attraction of female *Coleomegilla maculata* (DeGeer) (Coleoptera: Coccinelidae) to fava beans EFN (*Vicia faba* L.) is reduced in the presence of pea aphid *Acyrthosiphon pisum* Harris (Hemiptera: Aphididae) prey.[45] Seagraves and Lundgren[46] found that the addition of prey to thiamethoxam-treated plants increased the survival of adult *Orius insidiosus* (Say) (Hemiptera: Anthocoridae), indicating that the bugs alter their diet as prey becomes available. However, prey availability did not affect the survival of nymphs, which were more sensitive to thiamethoxam than adults. Similarly, Gontijo *et al.*[47] reported lethal and sublethal effects on *O. insidiosus*, when the insects fed on sunflower EFN contaminated with thiamethoxam-treated seeds.

Additionally, another hypothesis that may explain the lower toxicity of thiamethoxam to *H. axyridis* compared with *C. externa* is the possibility of lady beetle resistance to neonicotinoids. Cases of neonicotinoid resistance have been reported, particularly metabolic resistance due to enhancement of the expression of cytochrome P450s.[7] Research has revealed that sucking insect pests (e.g. *F. fusca* and *A. gossypii*) can cause severe damage in cotton fields grown from seeds treated with neonicotinoids, indicating the development of resistance.[48–50] In addition, neonicotinoid-treated seeds have been associated with increased two-spotted spider mite *Tetranychus urticae* Koch (Acari: Tetranychidae) infestations in seedling cotton.[51] Populations of natural enemies often exposed to pesticides may exhibit resistance in the same way as insect pests.[52,53] Barbosa *et al.*[54] found that *Hippodamia convergens* Guérin-Méneville (Coleoptera: Coccinellidae) populations' resistance to pyrethroid lambda-cyhalothrin and organophosphate dichrotophos is associated with extensive cotton crop sites wherein insecticides are frequently applied. Gontijo *et al.*[55] also associated the knockdown effect of thiamethoxam applied to soybean seeds on *Podisus nigripinus* (Dallas) (Hemiptera: Pentatomidae) with a probable degree of resistance of stink bugs to neonicotinoids.

Negative effects of thiamethoxam seed treatment on lady beetle were also reported by Moscardini *et al.*[35] who verified that the consumption of sunflower EFN from plants grown from treated seeds had sublethal and transgenerational effects on the biology of *C. maculata* and *H. convergens*. Moser and Obrycki[56] also observed that *H. axyridis* larvae exposed to maize seedlings treated with neonicotinoids thiamethoxam and clothianidin had neurotoxic disorders such as loss of coordination, reeling and inability to walk. By contrast, recommended doses of thiamethoxam for the control of cotton aphid *A. gossypii* were selective to *Cycloneda sanguinea* (Linnaeus) (Coleoptera: Coccinellidae) when the lady beetle was

Table 4. Life history parameters of *Harmonia axyridis* after adults fed on cotton extrafloral nectar of plants grown from thiamethoxam-treated seeds

		Seed treatment		Stats		
	Parameter	Untreated	Thiamethoxam	Critical value	df	*P*-value
Adults exposed (F0)	Preoviposition period (d)	3.8 ± 0.2	4.1 ± 0.3	147.5[b]	17, 18	0.86
	Fecundity*(eggs female^{-1})	116.2 ± 11.8	112.1 ± 17.8	0.2[a]	33	0.85
	No. clutches laid in 10-days	5.4 ± 0.4	5.3 ± 0.5	0.1[a]	33	0.91
	Fertility (egg hatching %)	60.9 ± 5.2	55.6 ± 6.7	0.6[a]	30	0.54
	Survival†(%)					
	Female	83.3 ± 7.8	95.8 ± 4.2			0.35[d]
	Male	66.7 ± 9.8	62.5 ± 10.1	0.0[c]	1	1.00
F1 generation	Larval period					
	Survival (%)	80.0 ± 7.1 a	58.0 ± 3.9 b	3.4[a]	8	0.03
	Development time (d)	10.9 ± 0.6	10.6 ± 0.2	0.5[a]	8	0.60
	Pupal period					
	Survival (%)	92.4 ± 3.1	95.6 ± 2.7	−0.7[a]	8	0.47
	Development time (d)	4.8 ± 0.1	4.6 ± 0.3	0.8[a]	8	0.42
	Adults					
	No. adults emerged	36	27			
	Sex ratio (proportion female)	0.44 ± 0.08	0.43 ± 0.10	0.1[c]	1	0.79
	Reproductive parameters					
	No. pairs mated	14	13			
	Preoviposition period (d)	9.4 ± 0.3	9.5 ± 0.3	−0.3[a]	12	0.79
	Fecundity*(eggs female^{-1})	38.8 ± 8.7	44.9 ± 8.0	−0.5[a]	13	0.31
	Fertility (egg hatching %)	100	90.1 ± 7.1	9.0[b]	5,6	0.32
	Survival†(%)					
	Female	100	100			–
	Male	100	100			–

Means (±SE) followed by different letters within rows were significantly different by (a) *t*-test, (b) Mann–Whitney, (c) chi-square or (d) Fisher's exact test ($\alpha = 0.05$). –, non-analyzed data.
*Number of eggs in 10-days or number of eggs in the first three clutches (F1 generation).
†Accumulated survival in the last evaluation day.

exposed *via* topical application and ingestion of contaminated prey.[57]

A recent study also reported non-target effects of thiamethoxam seed treatment *via* EFN consumption by parasitoids such as *Lysiphlebus testaceipes* (Cresson) (Hymenoptera: Braconidae).[58] Similarly, Stapel *et al.*[59] found that the consumption of cotton EFN contaminated with neonicotinoid imidacloprid by *Microplitis croceipes* Cresson (Hymenoptera: Braconidae) reduced the longevity and host foraging ability of the wasps. In cotton fields, imidacloprid and thiamethoxam seed treatment reduced the abundance of beneficial arthropods (~ 60%), particularly *Chrysoperla* spp. and coccinelids, with a population decrease after application of higher doses.[10] *Chrysoperla* spp. and *Nabis americoferus* (Carayon) (Hemiptera: Nabidae) were also less abundant in soybean fields with neonicotinoid seed treatments.[46] Similarly, Douglas and Tooker[60] used meta-analysis to conclude that neonicotinoids applied to seeds have negative effects on the abundance of arthropods' natural enemies. According to Zhang *et al.*[9] the lower abundance of natural enemies in cotton fields with neonicotinoid seed treatment is attributed to low prey density in the seed-treated plots. This can result in a failure to attract natural enemies, besides causing their mortality by consumption of cotton EFN containing insecticides.

In summary, our results indicate that thiamethoxam applied to cotton seeds is harmful to both *C. externa* and *H. axyridis*. However, lady beetles appear to be less sensitive to thiamethoxam than lacewings. These results show that systemic insecticides applied as seed treatment may not be safe for the conservation of natural enemies, contradicting the concept of ecological selectivity. Further studies with systemic insecticides applied to seeds are necessary for risk assessment in non-target organisms. Moreover, future studies should focus on assessing the potential risks to natural enemies' ecological services.

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